

THE INFLUENCE OF SEXUAL HORMONES ON THE ERYTHROCYTE
COUNT AND HEMOGLOBIN LEVEL IN CHICKENS

by

THEODORE LOUIS CRUSH

B. A., Ohio Wesleyan University, 1948

A THESIS

submitted in partial fulfillment of the

requirements for the degree

MASTER OF SCIENCE

Department of Zoology

KANSAS STATE COLLEGE
OF AGRICULTURE AND APPLIED SCIENCE

1949

Docu-
ment
LD
2668
74
1949
C71
C.2

TABLE OF CONTENTS

| | |
|-----------------------------|----|
| INTRODUCTION | 1 |
| MATERIALS AND METHODS | 3 |
| RESULTS | 6 |
| CONCLUSIONS | 24 |
| DISCUSSION | 27 |
| SUMMARY | 32 |
| ACKNOWLEDGMENTS | 33 |
| LITERATURE CITED | 34 |

INTRODUCTION

Since the turn of the century, there has been an ever increasing search for facts concerning the function, activity, and pathological influences of the endocrine glands upon the blood picture of the higher animals. One of the many significant observations recorded was that in most all mammals and birds, the erythrocyte count and hemoglobin concentration of males are higher than those of females.

The earliest observation of this difference that could be located in the literature was made in 1909 by Jolly (as reported by Finklestein, 1944) who found that a decrease of the erythrocyte count occurred in specific individuals that were castrated. These phenomena were further substantiated by Blacher (1926) who noted that the number of erythrocytes and quantity of hemoglobin were greater in the cock than in the hen. He also found that this dimorphism was more pronounced in the fowl than in man and could be observed as early as the third month after hatching. Furthermore, after castration the number of erythrocytes and percentage of hemoglobin had diminished in males while in females diminution was insignificant. Further studies by Juhn (1930) showed that in sexually immature chickens the erythrocyte count did not present a sex-based difference, but that upon approaching maturity the male erythrocyte count exceeded that of the female by approximately 30 per cent. On the other hand, castrated males showed a count very nearly equivalent to that of females. This latter observation was similar to results previously reported by Blumel (1928) while working with rats.

Further investigation into the sex gland-blood picture relationship showed that by the removal of an ovary of a fowl there was an immediate increase in the erythrocyte count (Blacher, 1926). This, as was demonstrated,

was due to the subsequent elaboration of testicular tissue. Investigations of this nature gave similar results but to a lesser degree in pigeons (Riddle, 1934). At the same time, Blacher showed that a sex gland when transplanted into a new host recovers the number of erythrocytes corresponding to its sex. Taking all these facts as a whole, it was agreed that sex glands while secreting their respective hormones do have a decided influence upon the blood picture of animals.

In the years that followed, greater stress was put upon new methods of approach including better control measures, assay methods, and ideal conditions to decrease discrepancies and error. With the production of synthetic sex hormones, these conditions were brought closer to reality. Testosterone, the principle male sex hormone compounded, was found, with proper administration and dosage, to increase the erythrocyte count in chickens (Domm et al. 1943), in rats (Finklestein et al. 1944), and in eumchoid male human beings (McCullagh and Jones, 1942). As a result of these studies, it was agreed there was direct stimulation of the hemopoietic system. On the other hand, estrogenic hormones reacted differently.

While studying dogs with normal blood pictures, Castrodale et al. (1941) reported that both sexes showed a decrease in erythrocyte number after repeated injections of stilbestrol in sesame oil. Likewise, Crafts (1941) with male rats, and Tyslovits and Dingemans (1941) with dogs of both sexes reported significant declines in erythrocyte number. Feuchtinger (1940) reported that female rats expressed only a slight anemia after injections of estrogens. However, in contrast to the previously mentioned reports on estrogens, Davis and Boynton (1941) found that their animals showed an increase in erythrocyte number with treatments of stilbestrol. In the same

year, Tyslovitz and Dingemans conducted an experiment to see what effect the two sex hormones had when given simultaneously. As a result, they reported that there was a decrease in erythrocyte count after castration in dogs. However, two years later, Dorn et al. (1943) found that by injecting capons with both sex hormones, there was a decided increase in erythrocyte count but that it was only 25 per cent of the increase obtained when only testosterone was injected.

Many workers agree that androgens and their counterparts stimulate the production of erythrocytes and increase hemoglobin concentrations while estrogens, in contrast, inhibit hemopoietic function. To test the validity of these conclusions, further experimental work was undertaken with special emphasis on the influence of estrogenic hormone.

MATERIALS AND METHODS

This experiment was undertaken in the Zoological laboratory of Kansas State College in January, 1949 and extended through June, 1949 under the supervision of Dr. E. H. Herrick. The animals under experimentation were single comb White Leghorn chickens selected from two separate shipments. The first shipment was received on October 1, 1948 from which 17 animals were selected. Of these 17, 16 were males which were caponized on December 9, 1948. The seventeenth was a female. The second shipment which was received on December 14, 1948 provided 8 females giving an overall total of 25 experimental animals.

In carrying out this experiment, the animals were subjected to intramuscular injections of synthetic sex hormones three times a week. Each week

their erythrocyte count and hemoglobin concentration were taken by securing blood from a pierced vein on the inner surface of the wing. In making the erythrocyte count, a one to two hundred dilution of Hayem's solution was made while the cells were counted on a Spencer Bright-Line hemacytometer. The hemoglobin concentration was made up as a one to two hundred and fifty dilution with one tenth normal hydrochloric acid. A Fischer Electro-Hemometer was employed for the reading of these concentrations which was in grams per hundred cubic centimeters.

The first of the series of experiments was carried out on 16 capons and 1 female. At the time of the first injection, the animals were approximately four months old. Prior to injections, however, the 16 capons were weighed to the nearest gram. On the basis of body weight, they were divided into two separate groups of 8 capons each. The birds in the first group were given three injections per week of testosterone propionate (Oreton), produced by the Schering Corporation. This hormone was dissolved in vegetable oil in the proportion of 25 milligrams per cubic centimeter of oil. Each capon received one tenth of a cubic centimeter of the solution or the equivalent of two and one half milligrams of active male hormone with each injection. During the complete injection period, they received thirty seven and a half milligrams of hormone.

The capons of the second group were injected with testosterone propionate in the same manner. In addition, they also received Theelin, a female sex hormone, produced by Parke-Davis and Company. This hormone was suspended in an aqueous solution with five milligrams of the active hormone in each cubic centimeter. With this, one half cubic centimeter of the suspension was given with each injection, thus administering two and one half milligrams of this

female sex hormone. One female treated with female hormone completed this phase of the experiments.

The second phase of the experiments was started on April 28, 1949 with eight females selected from the second shipment of animals. Of these eight, two acted as controls, receiving no hormones, but experiencing the same living conditions as the remaining six. These remaining six were weighed and then over a period of five weeks were injected with diethylstilboestrol, a synthetic compound with estrogenic properties, produced by Merck and Company. Five milligrams in each cubic centimeter of vegetable oil made up this dilution of which one-tenth of a cubic centimeter or one-half of a milligram was administered at each injection.

The third phase of the experiments, beginning on May 1, 1949, consisted of eight caesons which were selected from the first phase. At the time this experiment started, these eight caesons had reached normalcy in regards to erythrocyte count and hemoglobin concentration. Therefore, it was possible to submit them to further treatment. Again, by selecting for weight, the animals were placed into two groups of four each. The first group received the same treatment as did those of group one, phase one. Likewise, the method of treatment for the second group of phase one was used in treating the second group of phase three.

Upon completion of each phase of experiments, the animals were again weighed. From the data obtained, it was possible to tabulate the performance of each individual animal under treatment. Furthermore, it was possible to evaluate group differences and method of treatment response. And lastly, it was possible to compare these results with those obtained in previous experiments.

RESULTS

The data recorded from this investigation were the result of 279 erythrocyte counts and the same number of hemoglobin concentration readings plus a record of body weights over the various experimental periods. A comparative analysis of data of individual animals was used when they presented a wider deviation from the mean than other individuals of the same group. However, while calculating mean data these deviations were given no consideration since all the animals of the individual groups had experienced identical experimental conditions. Finally, the mean data of each group were incorporated into this report in graphic and tabular form plus the tabulation of percentage change of erythrocyte count, hemoglobin concentration, and body weight, in Figs. 1-7, Tables 1-7, and Table 8, respectively.

The first phase of the experiment consisted of a group of eight capons that were injected with male sex hormone. Prior to the injection period these animals showed a mean erythrocyte count of 2,404,000. However, after administration of three injections during the first week of the injection period, which extended over a period of four weeks, the mean count showed an increase of 30,600 cells. This rapid increase persisted for the following week when the mean count reached 3,050,000. At this point, although injections were maintained, the count rose less rapidly, when at the end of the fourth week a count of 3,350,000 was recorded. This was an increase of only 12,000 cells per animal as compared with an increase of 18,000 cells between the second and the third week. Injections were then discontinued, but the weekly erythrocyte counts were maintained. As a result, it was noted that the cell count began to decrease, not as rapidly as it had increased, but sufficiently

enough that by the end of the eleventh week, the mean count was down to 2,570,000 cells (Fig. 1). This rise and fall of the number of cells showed a percentage change of 39.00 which was approximately seven per cent less than a count of 3,500,000, normally found in males.

Pre-injection hemoglobin concentration was recorded at 10.26 grams per 100 cc of blood. As injections began, this level showed a decided increase just as did the erythrocyte count, reaching its maximum level of 12.37 at the end of the fourth week of injections. Here, too, upon discontinuation of injections, the concentration began to decrease less rapidly than it had increased. However, unlike the erythrocyte count, the mean concentration at the end of 11 weeks was identical to the mean pre-injection level. In no instance did the termination level of each individual vary more than 0.25 gm from the pre-injection level. The percentage change of hemoglobin concentration for this group of animals was 16.25. This figure, however, does not represent the percentage increase of all the animals since capons 1370 and 1382 showed an increase of only 10.73 and 10.72 per cent, respectively. No explanation can be offered as to why this occurred since the erythrocyte count of these animals was within two percentage points of the mean percentage change of 39.00.

Eight capons which received injection of both male and female hormones made up Group 2. A mean erythrocyte count of 2,446,000 was recorded for this group prior to the first injection. Due to a misunderstanding this group received only female hormone for the first two injections and male and female hormones for the third injection of the first week. The count taken at this time showed an increase of approximately 21,000 cells per animal. However, with subsequent injections of male and female hormones, the mean count rose to

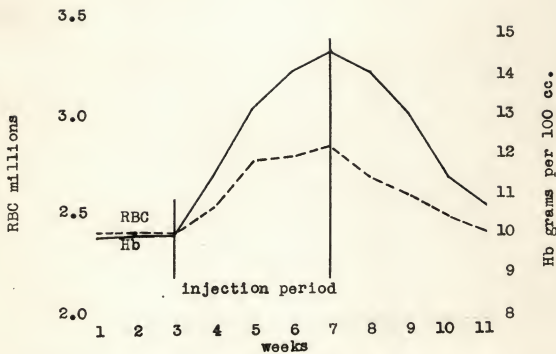


Figure 1. Group 1; Eight capons with male hormone.

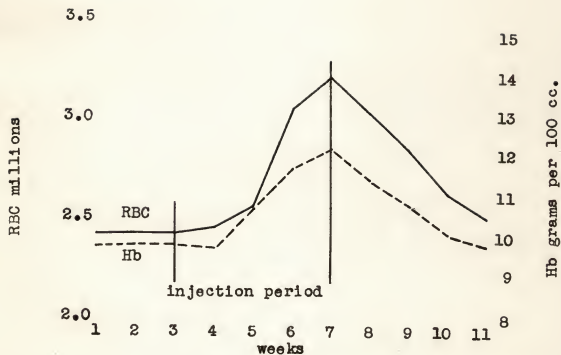


Figure 2. Group 2; Eight capons with male and female hormones.

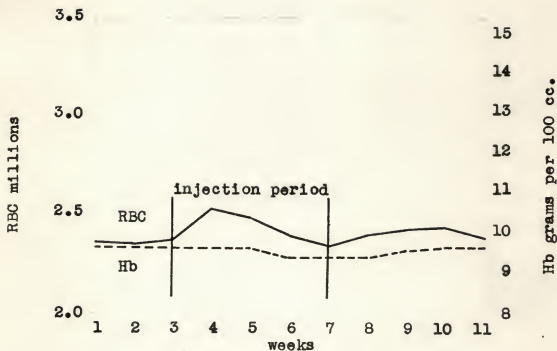


Figure 3. Group 3; One female with female hormone.

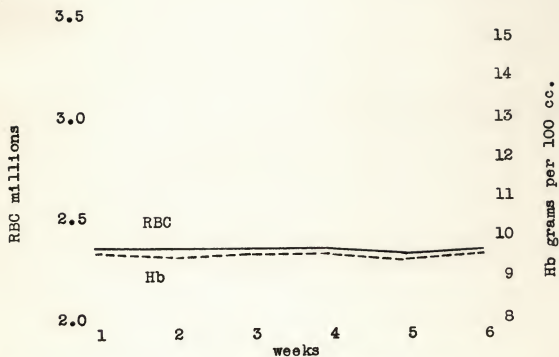


Figure 4. Group 4; Two normal female controls.

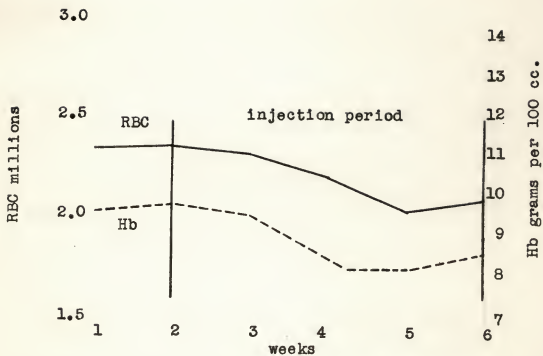


Figure 5. Group 5; Six females with female hormone.

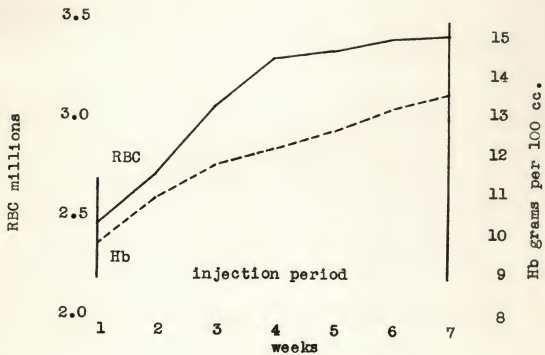


Figure 6. Group 6; Four capons with male hormone.

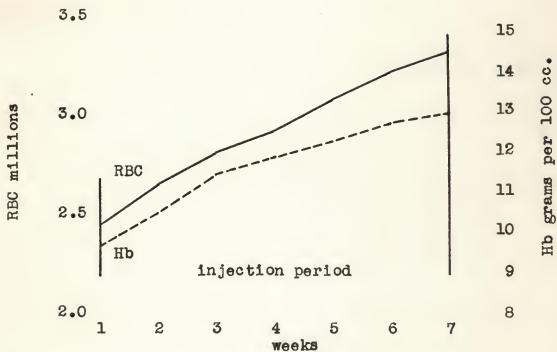


Figure 7. Group 7; Four capons with male and female hormone.

Table 1. Mean weekly data for eight capons injected with male hormone.

| Week | RBC | Hb |
|------|-----------|-------|
| 1 | 2 400 000 | 10.30 |
| 2 | 2 410 000 | 10.25 |
| 3 | 2 410 000 | 10.25 |
| 4 | 2 710 000 | 10.78 |
| 5 | 3 050 000 | 11.93 |
| 6 | 3 230 000 | 12.06 |
| 7 | 3 350 000 | 12.37 |
| 8 | 3 250 000 | 11.52 |
| 9 | 3 040 000 | 11.11 |
| 10 | 2 710 000 | 10.62 |
| 11 | 2 570 000 | 10.23 |

Table 2. Mean weekly data for eight capons injected with male and female hormones.

| Week | RBC | Hb |
|------|-----------|-------|
| 1 | 2 440 000 | 9.93 |
| 2 | 2 450 000 | 9.96 |
| 3 | 2 400 000 | 9.96 |
| 4 | 2 470 000 | 9.88 |
| 5 | 2 590 000 | 10.84 |
| 6 | 3 040 000 | 11.81 |
| 7 | 3 215 000 | 12.37 |
| 8 | 3 040 000 | 11.53 |
| 9 | 2 870 000 | 10.87 |
| 10 | 2 640 000 | 10.28 |
| 11 | 2 510 000 | 9.93 |

Table 3. Weekly data for one female injected with female hormone.

| Week | RBC | Hb |
|------|-----------|------|
| 1 | 2 380 000 | 9.75 |
| 2 | 2 370 000 | 9.75 |
| 3 | 2 380 000 | 9.75 |
| 4 | 2 540 000 | 9.75 |
| 5 | 2 500 000 | 9.75 |
| 6 | 2 410 000 | 9.50 |
| 7 | 2 360 000 | 9.50 |
| 8 | 2 410 000 | 9.50 |
| 9 | 2 430 000 | 9.66 |
| 10 | 2 450 000 | 9.75 |
| 11 | 2 400 000 | 9.75 |

Table 4. Mean weekly data for six females injected with female hormone.

| Week | RBC | Hb |
|------|-----------|------|
| 1 | 2 380 000 | 9.68 |
| 2 | 2 380 000 | 9.80 |
| 3 | 2 340 000 | 9.58 |
| 4 | 2 200 000 | 8.25 |
| 5 | 2 050 000 | 8.26 |
| 6 | 2 100 000 | 8.52 |

Table 5. Mean weekly data for two female controls.

| Week | RBC | Hb |
|------|-----------|------|
| 1 | 2 380 000 | 9.75 |
| 2 | 2 370 000 | 9.62 |
| 3 | 2 380 000 | 9.70 |
| 4 | 2 380 000 | 9.75 |
| 5 | 2 370 000 | 9.58 |
| 6 | 2 380 000 | 9.62 |

Table 6. Mean weekly data for four capons injected with male hormone.

| Week | RBC | Hb |
|------|-----------|-------|
| 1 | 2 470 000 | 9.87 |
| 2 | 2 730 000 | 11.12 |
| 3 | 3 070 000 | 11.81 |
| 4 | 3 310 000 | 12.31 |
| 5 | 3 340 000 | 12.75 |
| 6 | 3 410 000 | 13.25 |
| 7 | 3 427 000 | 13.68 |

Table 7. Mean weekly data for four capons injected with male and female hormone.

| Week | RBC | Hb |
|------|-----------|-------|
| 1 | 2 460 000 | 9.79 |
| 2 | 2 660 000 | 10.68 |
| 3 | 2 810 000 | 11.52 |
| 4 | 2 940 000 | 12.00 |
| 5 | 3 080 000 | 12.37 |
| 6 | 3 230 000 | 12.75 |
| 7 | 3 310 000 | 13.06 |

Table 8. Data for individual birds on changes in RBC, Hb, and weight.

| Bird | Sex | Hormone | RBC | Hb | Weight |
|------|--------|---------------------------|----------|-------|--------|
| | | | Per cent | | |
| 1362 | capon | androgenic | 42.83 | 16.37 | 13.73 |
| 1366 | capon | androgenic | 42.50 | 22.50 | 7.95 |
| 1367 | capon | androgenic | 36.40 | 20.00 | 22.65 |
| 1370 | capon | androgenic | 41.10 | 10.73 | 12.76 |
| 1374 | capon | androgenic | 40.78 | 27.50 | 17.21 |
| 1376 | capon | androgenic | 37.50 | 26.95 | 20.55 |
| 1377 | capon | androgenic | 40.67 | 22.50 | 10.46 |
| 1382 | capon | androgenic | 37.63 | 10.72 | 16.52 |
| 1363 | capon | androgenic and estrogenic | 26.98 | 21.90 | 18.00 |
| 1364 | capon | androgenic and estrogenic | 30.89 | 13.94 | 15.57 |
| 1365 | capon | androgenic and estrogenic | 38.79 | 28.94 | 6.15 |
| 1368 | capon | androgenic and estrogenic | 29.88 | 30.76 | 15.01 |
| 1369 | capon | androgenic and estrogenic | 31.45 | 42.84 | 13.87 |
| 1378 | capon | androgenic and estrogenic | 34.02 | 25.00 | 17.78 |
| 1383 | capon | androgenic and estrogenic | 30.45 | 20.00 | 30.51 |
| 1361 | capon | androgenic and estrogenic | 30.34 | 25.73 | 14.87 |
| 1360 | female | estrogenic | 7.15 | 2.73 | 13.56 |
| 1384 | female | estrogenic | 11.16 | 18.18 | 3.14 |
| 1385 | female | estrogenic | 17.82 | 21.87 | 2.97 |
| 1386 | female | estrogenic | 20.69 | 15.15 | 2.85 |
| 1387 | female | estrogenic | 19.50 | 21.87 | 4.51 |
| 1388 | female | estrogenic | 9.60 | 25.00 | 4.08 |
| 1392 | female | estrogenic | 20.30 | 21.87 | 4.00 |
| 1390 | female | none | | | 9.99 |
| 1391 | female | none | | | 6.38 |
| 1367 | capon | androgenic | 38.64 | 37.50 | 3.12 |
| 1370 | capon | androgenic | 41.22 | 35.89 | 3.91 |
| 1377 | capon | androgenic | 40.72 | 43.58 | 6.84 |
| 1383 | capon | androgenic | 41.46 | 37.50 | 3.88 |
| 1362 | capon | androgenic and estrogenic | 36.62 | 39.47 | 3.21 |
| 1365 | capon | androgenic and estrogenic | 33.46 | 33.33 | 3.50 |
| 1366 | capon | androgenic and estrogenic | 32.38 | 31.98 | 3.80 |
| 1378 | capon | androgenic and estrogenic | 35.34 | 29.26 | 4.60 |

2,590,000 at the end of the second week, with the greatest increase recorded during the third week when the mean count was established at 3,040,000 or an increase of approximately 450,000 cells per bird. This large increase was assumed to be caused by the male hormone gaining prestige over the female hormone that was singularly administered in the first two injections. In the week following this rapid increase, the mean count for the group reached its peak of 3,315,000 cells. Cessation of injections at this point caused a gradual decrease in erythrocyte count gathering its greatest momentum between the third and fourth week following the discontinuation of injections, when the recorded decrease in number was 230,000 cells per animal. At the end of 11 weeks, the mean count had fallen to 2,510,000, just 64,000 cells more than the pre-injection level. Taking the group as a whole, their mean erythrocyte count had increased 31.43 per cent. This average represented very closely individual averages of the group excepting bird 1365, which had an average increase of 38.79 per cent. The increase in capon 1365 was probably due to its cell-making processes since it recorded a pre-injection count of 2,326,000. Furthermore at the last determination, the count of this bird was 70,000 cells below the mean count of the group.

An initial average of 9.95 grams per 100 cc of blood was recorded for hemoglobin concentration of this group. Following the first week of injections, the mean concentration decreased 2.88 grams. This decrease was considered insignificant since in the subsequent week the level more than compensated for the decrease while a level of 10.84 was recorded. During the remaining injection period, this level gradually increased reaching a peak of 12.37. The first week after discontinuation of injection, the mean concentration was 11.53, maintaining a gradual decline until at the conclusion of the experiment

the average concentration was 9.93 assuming a level comparable to the pre-injection mean. The animals presented a wide variance in their percentage increase of hemoglobin concentration ranging from 13.94 to 42.84 per cent. As a group, an average increase of 24.32 per cent was recorded suggesting that treatment responses to male and female hormone given simultaneously are not as active as those responses stimulated by male hormone alone. This was further substantiated by the fact that the level of group two did not reach the proportions of that of group one (Fig. 2).

One female, 1360, injected with female hormone completed the first phase of the experiments. Pre-injection erythrocyte count showed a mean of 2,376,000. Upon administration of hormone, the count began to rise as was indicated by the first reading. This reading showed a count of 2,540,000. However, in the following week this elevation of count was succeeded by a gradual decrease in the number of cells when at the end of the fourth and final week of injections, the count was recorded at 2,360,000 which was only 20,000 cells less than the pre-injection mean. Post-injection counts showed a gradual rise to 2,450,000 cells at the end of the tenth week followed by another decrease at the eleventh week when the count was 2,400,000. Calculating percentage change in terms of the largest number of cells as compared with the smallest number of cells recorded, it was found to be a decrease of 7.15 per cent. Furthermore, a smaller percentage change of 0.84 per cent was recorded when the pre-injection mean was compared with the least number of cells reported. This suggested that the estrogenic hormone involved could have influenced the blood picture in two ways. First, that it stimulated cell production at the time of the primary injection or secondly, that it repressed cell formation when the volume of hormone had been increased in the animal's body as a result of repeated

injections.

Hemoglobin concentration in this animal showed very little change over the 11 week period as it varied no more than 0.25 gram which was recorded between the sixth and eighth week of the experiment. This, in terms of per cent, was only 2.73.

The second phase of the experiment consisted of six females treated with female hormone (Fig. 5) and two untreated ones. A mean erythrocyte count of 2,380,000 was recorded for these animals at the beginning of the experiment that later produced a depressed curve with a low mean count of 2,050,000. Birds 1386 and 1392 approached anemic conditions when their individual counts decreased to 1,980,000 and 1,970,000, respectively, during the third week of injections. However, at the end of the fourth week their erythrocyte counts had reached the mean for the group at 2,100,000 or higher. The experiment was terminated at this point since the primary interest was in the estrogenic influence on the erythrocyte count and hemoglobin level during the initial weeks of injections. Although, the count was still low at the conclusion of the experiment, it soon reached normalcy. Indications of this fact were brought out when the mean count of 2,100,000 was established during continued injections of the fourth week showing a mean increase of 50,000 cells per bird over the previous week. As a group, the mean erythrocytic change showed a decrease of 16.09 per cent which represented the individual percentage change of all the birds within 4.00 per cent except bird 1388 with a percentage decrease of only 9.60 per cent.

The group had an initial mean hemoglobin concentration of 9.74 grams. Upon the administration of hormone, the mean hemoglobin level decreased to 9.58 the first week followed by a mean level of 8.25 for the subsequent second

and third weeks of injections. The fourth and final week of injection reported a mean concentration of 9.52 indicating that the animals' body processes had overcome the estrogenic effects and were approaching normalcy. The group showed a mean percentage change of hemoglobin greater than that of their mean erythrocyte change with a calculated 18.08 per cent as compared with 16.09 per cent, respectively. No significant individual changes were reported for the group.

Two of the eight animals used in this phase of experiments acted as controls. They received no hormones, but were submitted to the same living conditions as those six receiving injections. The mean initial erythrocyte count for these animals was established at 2,385,000 and persisted for the first four weeks varying only slightly during the fifth week when it dropped to 2,370,000. The sixth and final week found the mean count at 2,380,000 (Fig. 4).

Mean hemoglobin concentration varied a little more than did the erythrocytic mean. However, the variation was of little or no significance since it did not exceed 0.23 gram throughout the six weeks of observations.

Eight castrons previously used in the first phase of the experiment that had reached normalcy constituted the third and final phase of the experiments. They were selected for weight and divided into two groups of four each (Groups 6 and 7). Group 6 received injections of male hormone with the procedure being the same as that which was used for Group 1 of the first phase of the experiment. Pre-injection counts showed a mean erythrocyte count of 2,475,000 for this group. Immediately following the first injection, the count began to increase rapidly. After three weeks of injections, the mean count had reached 3,310,000. At this point, the increase was more gradual when at the

end of seven weeks, the count attained a peak of 3,427,000. As can be noted in Table 6, this peak was only 25,000 cells greater than the previous mean reading indicating that a leveling off point had been attained. Furthermore, the mean count of this group showed a recorded peak of some 70,000 cells greater than did group one of the first phase. This was attributed to the fact that these animals were more mature at the time of this experiment suggesting that their hemopoietic responses were more keen and reactive than they had been previously. This group showed a mean erythrocyte increase of 40.52 per cent and all four birds were within two per cent of the average.

Mean weekly hemoglobin concentration of 9.87 grams was recorded for this group prior to the injection period. After six weeks of injections, the mean concentration had reached a peak of 13.68 with the greatest increase reported during the first week of injections when the mean concentration rose from 9.87 to 11.12. The mean percentage of increase in hemoglobin concentration was 37.58 for the four members of this group, each member showing an individual increase comparable to the mean. No correlation was established between erythrocyte count and hemoglobin increase since three members showed a larger increase in erythrocyte count over hemoglobin concentration while one member was just the reverse.

During this phase of the experiment, the four capons of Group 7 received injections of both male and female hormone. The mean erythrocyte count increased from a pre-injection level of 2,460,000 to 3,310,000 following six weeks of injections. The increase was the greatest during the first week of injections, slowing down through the second and third weeks and picking up during the next two. The final week showed a mean increase per animal of 82,000 cells as compared with 150,000 cell increase of the previous week.

The group varied no more than two per cent from the mean percentage increase of 34.45.

A high of 13.06 grams was established for the mean hemoglobin concentration of this group at the end of seven weeks after emerging from a pre-injection low of 9.79. The results showed that the greatest increase was during the first week of injections only to rise less gradually throughout the remaining period. The percentage change for the individuals varied from 29.26 to 39.47, with an average of 33.40 increase for the group.

All of the animals used in this experiment were weighed prior to the pre-injection counts and again at the conclusion of each experimental phase. Their percentage of increase was tabulated and included in this report in Table 8. No correlation was established between these weights and erythrocyte count or hemoglobin concentration.

CONCLUSIONS

A thorough analysis of the data recorded from this investigation presented a number of significant conclusions. The first of these was that testosterone propionate was the stimulating factor that caused an increase in the erythrocyte count and hemoglobin concentration. This phenomenon was evident in those two groups that received only male sex hormones, namely Group 1 and Group 6. Prior to the experimental period these animals were castrated. As a result, their erythrocyte count and hemoglobin level decreased to one million and five grams per hundred cubic centimeter below that of an average normal male, respectively. Furthermore, secondary sexual characteristics were observed to diminish as a result of this castration. However, with administration of testosterone

propionate to these capons, their erythrocyte count and hemoglobin concentration increased to the extent as to approach that of a normal male. Also a physiological restoration of male characteristics was observed. Furthermore, upon discontinuation of these injections, the erythrocyte count and hemoglobin concentration diminished sufficiently as to approximate that of the pre-injection level. Conclusions then reached were; since testosterone is secreted by the testes, removal of these testes decreased the volume of testosterone secreted as to proportionally decrease the erythrocyte count and the hemoglobin concentration. In addition, when the normal volume of testosterone is replenished, the erythrocyte count and hemoglobin concentration approach the male level. And lastly, when this volume is allowed to diminish, by discontinued injection, the erythrocyte count and hemoglobin concentration decrease to the capon level. Therefore, the observable increases in erythrocyte count and hemoglobin concentration were directly due to the administration of testosterone propionate.

The second outstanding conclusion was supplied by Groups 2 and 7 in that estrogen repressed the erythropoietic action of testosterone propionate. These two groups, unlike the two previously mentioned, received both testosterone and estrogen injections. Evidence supporting these phenomena was observed by the fact that the degree of mean increase of erythrocyte count and hemoglobin concentration for these groups was much less than that of those groups that received only testosterone. Estrogen was considered the repressing factor because it was the only variable introduced. Furthermore, it was concluded that estrogen did not exert its influence upon the erythropoietic action of testosterone until after it was well established. This occurred specifically in Group 5 during the third week of injections when the

acceleration in erythrocyte increase was slowed down to approximately half. In the second group of phase one estrogen had a foothold from the start. Thus, this particular conclusion is not exemplified. Nevertheless, it was reasonable to decree that estrogen does repress as well as express itself at a definite time on the erythropoietic action of testosterone propionate.

In reference to estrogen, another conclusion was reached in that it did not alter the blood forming processes but that its presence in excess of a normal volume of estrogen caused an imbalance of the endocrine system. This conclusion was established from the data secured from the second phase of the experiment, and the one female under observation in the first phase. The second phase consisting of six females showed a decided decrease in erythrocyte count and hemoglobin concentration upon administration of estrogen which, at first, might have nullified this conclusion except for the fact that the erythrocyte count and hemoglobin concentration were increasing although injections were still administered. Further evidence provided by female 1360 showed a rise in erythrocyte count and hemoglobin concentration upon administration of estrogen followed by a decrease. During the first post-injection week, the erythrocyte count and hemoglobin level rose higher than the pre-injection level and remained that way throughout the remaining portion of the experiment. It was concluded that these animals experiencing such a condition were able to overcome and correct this abnormality caused by estrogen even while the estrogen was present in excess of normal estrogenic volume in the animal.

It is a well established fact that the anterior portion of the pituitary governs the amount of gonadotropic hormone produced. Likewise, it itself exercises an erythropoietic activity. With this in mind, it was reasonable

to conclude that estrogen exercises its influence by its repression of the anterior pituitary. This conclusion was brought out in the case of those groups that received both testosterone propionate and estrogen as compared with those groups that received testosterone propionate only. The former groups produced an increase that was less than the latter. No natural gonadotropic hormone was involved since the animals were castrons. Therefore, estrogenic influence on the blood picture by the reduction of erythropoietic activity in the anterior pituitary could well be the explanation of this phenomenon.

DISCUSSION

Research in the field of Endocrinology, as in many fields of science, has used a multitude of various experimental methods and likewise many different animals in seeking the how and why of the endocrine system. With the aid of many of these methods, biologists as well as all these scientists who have experimented directly with the endocrine system have agreed that testosterone propionate initiates an increase in erythrocyte number and hemoglobin concentration. Many theories and hypotheses have been advanced by workers in an effort to explain just how these phenomena occur, however, no experimental proof has been secured that fully substantiates their reasoning.

Of the many theories and hypotheses advanced, two have held far wider favor than the rest. These two were formulated by McCullagh and Jones (1942) who were of the opinion that androgens either exerted their influences on the blood forming elements or on the metabolic rate of the individual. Both explanations have received considerable amount of supporting evidence. The

former explanation was substantiated by Vollmer and Gorden (1941) and Vollmer et al. (1942), who made anaylitic examinations of the red bone marrow of rats treated with testosterone. They found that testosterone caused a hyperplasia of the erythroblastic elements. Furthermore, testosterone seemed to prevent the hypoplasia of erythroplastic tissue that usually resulted from hypophysectomy. As a result of the findings of Finklestein et al. (1944), Wentworth et al. (1940) and Rubenstein and Solomon (1941), it was assumed that the proliferation of the red bone marrow might be only a special manifestation of the general stimulation of somatic growth by testosterone. In support of the metabolic rate theory, Steinglass et al. (1941) and Crafts (1946) found that an increase in the basal metabolic rate in rats created an increased oxygen demand. As a result, there was an increase in erythrocyte number and hemoglobin concentration due to response of the erythropoietic system to this demand. Further increases in the basal metabolic rate of rats, caused by testosterone, were reported by Sandiford (1941), Kenyon et al. (1940), and Jones et al. (1941). Several workers, such as Meyer and Danow (1942) reported that they had observed no changes in metabolic rate in rats after castration or upon the injection of testosterone while Adams and Shevket (1929) after reporting a significant increase in metabolic rate claimed that this increase was due to a chain reaction with the key influence being exerted through the thyroid or pituitary glands which have been proven to exercise a decided effect upon basal metabolic rate.

Other experiments that were undertaken in an effort to find the dominating factors as to how testosterone increased erythrocyte number and hemoglobin concentration were made by Finklestein et al. (1944) who recorded the regeneration of erythrocytes and hemoglobin in rats after an artificially

induced anemia. They found that testosterone accelerated the erythrocyte regeneration in normal males and females, hypophysectomized males and females, and castrated males. However, the hemoglobin regeneration processes showed no acceleration in many of the animals. In addition Crafts (1946) experimenting with castrated rats reported that testosterone increased the erythrocyte count and hemoglobin concentration while discontinuation of testosterone injections caused both elements to return to pre-injection level. Similar observations were reported by McCullagh and Jones (1942) with eunuchoid men, Juhn and Domm (1930), and Stein and Jacobsen (1944) with golden hamsters, Taber et al. (1943) with chickens, and Vollmer et al. (1942), Steinglass et al. (1941), Vollmer and Gordon (1941) with rats.

The results of this experiment do not elaborate or confirm the methods of action of testosterone on the blood picture. However, it did support the findings of past investigators that testosterone increased the erythrocyte count and hemoglobin concentration as was exhibited by Groups 1 and 6.

As much or even more controversy has arisen over the method of influence that estrogen exerts on the blood picture. Castrodale et al. (1941) reported that upon administration of estrogen to dogs of both sexes, there developed an anemic condition to such a degree as to have proved fatal. This condition was also reported by Tyslovitz and Dingemans (1941) after a previous observation was made to the effect that only a slight decrease in erythrocyte number and hemoglobin concentration was exhibited in similar animals by estrogen. Additional experiments that resulted in detrimental effects on the blood picture by estrogen were performed by Finklestein et al. (1944) on anemic male, female, and castrate rats. Their results showed that the erythrocytic regenerative processes were inhibited by estrogen. Crafts (1941)

and Vollmer and Gordon (1941) stated that monkeys and dogs of both sexes exhibited a definite decrease in erythrocyte number and hemoglobin concentration. In all the cases mentioned thus far, in regard to estrogenic effects on the blood picture, it is indicated that the hemoglobin paralleled the degree of change in erythrocyte number. However, Robscheit-Robbins and Whipple (1941) reported that a severe anemia of 6.00 grams per cent of hemoglobin in dogs, as a result of estrogen, initiates a maximal stimulus for the production of new hemoglobin. Thus, the volume of hemoglobin present increased while the erythrocyte number remains constant. In direct contrast to these experiments performed, Davis and Boynton (1941) observed that stilbestrol exercised a beneficial effect on the hemopoietic system of human beings of both sexes. No other experiment has ever substantiated these results. However, Feuchtinger (1940) found that human beings while under the influence of estrogen therapy, demonstrated a decrease in erythrocyte count but that this count returned to normal even though the administration of estrogen was maintained. He reported that this condition was also prevalent in female rats and rabbits and particularly in rats during each period of estrus. Groups 3 and 5 of this experiment substantiated the results of the previously mentioned conditions but to a lesser degree.

From what has been observed of estrogenic influence on the blood picture, past workers have concluded that the method of influence is exerted in three possible ways. First, that estrogen created an antagonistic effect toward testosterone. As a result, an inhibition of the erythropoietic processes was effected. This hypothesis was supported in particular by Taber et al. (1943) who observed that estrogen caused a greater decrease in erythrocyte number in males than in females or castrons. Secondly, that estrogen acted directly

on the blood picture. Probably more investigators support their view than all the rest, since the basis for their belief has substantial support. Glass (1943), Heller and Thayer (1948), Tyslowitz and Hartman (1941), Crafts (1941), and Castrodale et al. (1941) all reported that estrogen acted directly on the blood stream because upon examination of red bone marrow a definite hypoplasia occurred as a result of estrogen therapy. Furthermore, that since estrogen caused a decrease in erythrocyte count and hemoglobin concentration of both sexes their assumption seemed all the more reasonable. Thirdly, Sherwood and Bowers (1936), Steinglass et al. (1941) supported the theory that estrogen exercised its influence on the blood picture by reducing the metabolic rate which in turn decreased the demand for erythrocytes by the individual.

Estrogenic influence on the blood picture, as concluded in this investigation, was determined by its action upon the anterior pituitary. The basis for this assumption began with the fact that workers, such as Pencharz (1940), Vollmer et al. (1942), Crafts (1941), observed that hypophysectomy created a degeneration of erythroblastic tissues as well as a decreased erythrocyte count. Furthermore, Beilly and Solomon (1940) and Rubenstein and Kurland (1940) reported that the anterior pituitary was definitely depressed by action of sex hormones. An excess of sex hormones was claimed to be the depressing factor upon the pituitary (Widelsberg and Ornstein, 1940). Nevertheless, it was reasonable to assume that the data obtained from Groups 2 and 7 readily illustrate this conclusion. These two groups received both testosterone and estrogen. The testosterone increased the erythrocyte count, yet by the action of estrogen on the anterior pituitary, the erythropoietic activity of this gland was reduced to the extent that the erythrocyte number did not reach the proportions of those groups of animals that received only testosterone, namely

Groups 1 and 6. Furthermore, in Groups 3 and 5 which were females that received estrogen, the decreases were not as significant as those reported by some workers due to the fact that the dosage used was minimal. As a result, the depressing ability of the estrogen on the anterior pituitary was such that the normal activity of the pituitary was able to overcome the estrogenic influence.

SUMMARY

The results recorded in this investigation and interpreted in the text of this thesis gave rise to the following conclusions:

1. Testosterone propionate initiated a definite increase in erythrocyte count and hemoglobin concentration of capons.
2. Capons that received both testosterone propionate and estrogen, the erythropoietic activity of testosterone was repressed by estrogen.
3. Estrogen expressed its greatest influence upon erythrocyte count and hemoglobin concentration only in capons who also received testosterone propionate.
4. Estrogen exhibited its influence later during the injection period when given simultaneously with testosterone propionate to capons.
5. Estrogens do not act directly upon the blood elements or those factors that form these elements.
6. The method of action upon erythrocyte number and hemoglobin concentration, as demonstrated by estrogen, was assumed to be through its influence upon the anterior pituitary and not on the blood forming elements directly.

ACKNOWLEDGMENTS

To Dr. E. H. Herrick, Professor of Zoology, Kansas State College, the author wishes to express his sincere thanks in supplying generous advice and assistance in carrying out this experiment and to Mrs. Lois Montague for her excellent technical assistance.

LITERATURE CITED

- Adams, A. Elizabeth and Fazile Shevket.
The normal blood picture of white rats and the changes in the picture following thyroid feeding. *Physiol. Zool.* 2:181-217, April, 1929.
- Beilly, Jacob S. and Samuel Solomon.
The inhibition of lactation postpartum with testosterone propionate. *Endocrinology.* 26:236-239, February, 1940.
- Blacher, L. J.
The influence of sexual hormones upon the number of erythrocytes and percentage quantity of hemoglobin by fowl. *Biologia Generalis.* 2:435-441, 1926.
- Blumel, Paul.
Castration and erythrocyte count. (in German). *Medical Clinics.* 24:1436-1452, 1928.
- Castrodale, Dante, Olga Bierbaum, Elson B. Helwig, and Cyril M. MacBryde.
Comparative study and the effects of estradiol and stilbestrol upon the blood, liver, and bone marrow. *Endocrinology.* 29:363-371, September, 1941.
- Crafts, Roger Conant.
The effect of endocrines on the formed elements of the blood. *Endocrinology.* 29:596-617, October, 1941.
- Crafts, Roger Conant.
Effects of hypophysectomy, castration, and testosterone propionate of hemopoiesis in the adult male rat. *Endocrinology.* 39:401-413, December, 1946.
- Davis, Edward M. and Melbourne W. Boynton.
Indications, clinical use, and toxicity of 4-4' dihydroxy-diethyl stilbene. *Jour. Clin. Endocrinology* 1:339-344, April, 1941.
- Downs, L. V., Elsie Taber, and David E. Davis.
Comparison of erythrocyte numbers in normal and hormone treated brown leghorn fowl. *Proc. Soc. Expt. Biol. and Med.* 52:49-50, 1943.
- Hidelsberg, Joseph, and Elliot A. Ornstein.
Observations on the continued use of male sex hormone over a long period of time. *Endocrinology.* 26:46-53, January, 1940.
- Feuchtinger, Oswald.
Follicle hormone and the blood picture. (in German). *Archives of Experimental Pathology and Pharmacology.* 196:644-670, 1940.

- Finklestein, Grace, Albert S. Gordan, and Harry A. Charriper.
The effect of sex hormones on the anemia induced by hemorrhage in the rat. *Endocrinology*. 35:267-277, October, 1944.
- Glass, S. J.
A case of myxedema with Macrocytic anemia successfully treated with thyroid and testosterone. *Jour. Clin. Endocrinology*. 3:421-425, July, 1943.
- Heller, V. G. and Rollin H. Thayer.
Chemical changes in the blood composition of chickens and monkeys fed synthetic estrogens. *Endocrinology*. 42:161-167, March, 1948.
- Jones, Reid, E. Perry McCullagh, D. Roy McCullagh, and G. H. Buckaloo.
Observation on the hypermetabolism induced by methyl testosterone. *Jour. Clin. Endocrinology*. 1:656-660, August, 1941.
- Juhn, Mary, and L. V. Domm.
The relation of gonadal condition to erythrocyte number in fowl. *Amer. Jour. Physiol.* 94:656-661, September, 1930.
- Kenyon, Allan T., Kathryn Knowlton, Irene Sandiford, F. C. Koch, and Gertrude Lotwin.
A comparative study of the metabolic effects of testosterone propionate in normal men and women and in eunuchoidism. *Endocrinology*. 26:26-45, January, 1940.
- McCullagh, E. Perry, and Reid Jones.
Effect of androgens on the blood count of men. *Jour. Clin. Endocrinology*. 2:243-251, April, 1942.
- Meyer, Arthur, E. and Helen Danow.
Influence of methyltestosterone on metabolism of normal, castrate, and thyroidectomized rats. *Proc. Soc. Expt. Biol. and Med.* 49:598-601, April, 1942.
- Meyer, Ovid O., Ethel W. Thewlis and Harold P. Rusch.
The hypophysis and hemopoiesis. *Endocrinology*. 27:932-944, December, 1940.
- Penchars, Richard I.
Effect of estrogens and androgens alone and in combination with chorionic gonadotropin on the ovary of the hypophysectomized rat. *Science*. 91(2371):554-555, 1940.
- Riddle, Oscar, and George E. Cauthen.
Erythrocyte number in young pigeons and its relation to heredity, growth, and metabolism. *Amer. Jour. Physiol.* 122:514-525, May, 1938.
- Riddle, Oscar, and Pela Fay Braucher.
Hemoglobin and erythrocyte differences according to sex and season in doves and pigeons. *Amer. Jour. Physiol.* 108:554-566, March, 1934.

- Robacheit-Robbins, F. S., and G. H. Whipple.
Hemoglobin production increases with severity of anemia. *Amer. Jour. Physiol.* 134:263-261, 1941.
- Rubenstein, H. S. and A. A. Kurland.
Effect of small doses of testosterone propionate on testis of albino rats. *Proc. Soc. Expt. Biol. and Med.* 44:168-169, 1940.
- Rubenstein, H. S. and M. L. Solomon.
The growth depressing effect of large doses of testosterone propionate in the castrate albino rat. *Endocrinology.* 28:112-116, January, 1941.
- Rubenstein, H. S. and M. L. Solomon.
The growth stimulating effect of small doses of testosterone propionate in the castrate albino rat. *Endocrinology.* 28:229-236, February, 1941.
- Sandiford, Irene, Kathryn Knowlton, and Allan T. Kenyon.
Basal heat production in hypogonadism in men and its increase by protracted treatment with testosterone propionate. *Jour. Clin. Endocrinology.* 1:931-944, December, 1941.
- Sherwood, T. C. and Linwood M. Bowers.
The effect of ovarian hormone on the basal metabolism of experimental hyperthyroid rat. *Amer. Jour. Physiol.* 115:645-650, May, 1936.
- Stein, Kathryn, and Barbara Jacobsen.
The effect of castration and cryorchidization on the blood picture of the golden hamster. *Anatomical Record.* 88:459, 1944. (abstract).
- Steinglass, Paul, Albert S. Gordon, and Harry A. Charriper.
Effect of castration and hormone on the blood picture of rats. *Proc. Soc. Expt. Biol. and Med.* 48:169, October, 1941.
- Taber, Elsie, David E. Davis, and L. V. Dunn.
The effect of sex hormones on the erythrocyte number in the blood of the domestic fowl. *Amer. Jour. of Physiol.* 138:479-487, February, 1943.
- Tyslovitz, R. and E. Dingemans.
Effect of large doses of estrogen on the blood picture of dogs. *Endocrinology.* 29:817-827, November, 1941.
- Tyslovitz, R. and Carol G. Hartman.
Influence of large doses of estrogen on the blood picture of rhesus monkeys. *Endocrinology.* 29:349-351, September, 1941.
- Vollmer, Erwin P., and Albert S. Gordon.
Effect of sex and gonadatropic hormones upon the blood picture of the rat. *Endocrinology.* 29:828, November, 1941.

Vollmer, E. P., Albert S. Gordon, and Harry A. Charipper.

Effects of hormones on erythropoiesis in the hypophysectomized rat.
Endocrinology. 31:619-628, 1942.

Wentworth, J. H., P. K. Smith, and W. W. Gardner.

The composition of bones of mice receiving estrogens and androgens.
Endocrinology. 26:61-67, January, 1940.

[illegible]

Dec 11 '59A